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Validation of speed of sound for the assessment of cortical bone maturity

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SUMMARY Bone changes its structural and physical properties during maturation. In order to validate ultrasound measurements with regard to their usefulness in assessing cortical bone maturation, speed of sound (SOS) data were compared with mechanical properties (elastic modulus, bending strength, and cortical surface hardness), density and water content. Thirty pig mandibles were selected from three different age groups. Rectangular specimens of the buccal cortical bone of the body of the mandible were prepared. SOS was measured with pulsed ultrasound at a frequency of 2 MHz in all three dimensions, bone mineral density (BMD) by quantitative computed tomography, breaking strength and apparent elastic modulus in a three-point bending test to failure, water content using the lyophilization technique, and micro-indentation hardness using a modified Vickers' technique.

While SOS in all three directions, BMD, surface hardness, and bending strength increased significantly ($P < 0.001$), bone tissue water content decreased significantly ($P < 0.001$) with age. Changes in the elastic modulus were not significant. Changes in SOS in the antero-posterior and apico-occlusal directions can be partly explained by BMD. In a bucco-lingual direction the increase is inadequately explained by the physical parameters investigated, and has possibly to be attributed to structural differences. Maturation of the mandible implies changes in its architectural organization, in material composition, and in the mechanical properties of cortical bone.

In vitro SOS measurements reflect different structural and physical properties that are all age dependent. It thus seems feasible that age-related changes in bone maturation could be monitored by SOS measurements.

Introduction

Certain bone quality parameters, such as tissue mineral density or calcium metabolism, have been shown to influence the biology of tooth movement (Midgett *et al.*, 1981; Bridges *et al.*, 1988). Stable orthodontic treatment is dependent on the biological system adapting to the tooth's new position by remodelling of the surrounding tissues. The extent of remodelling is influenced primarily by physiological cellular metabolism in the alveolar bone, and also by the status of bone maturation. The latter includes a large number of parameters that alter constantly during growth and ageing. Although macro- and micro-anatomical changes in different regions of the mandibular hard tissue have been established, particularly alveolar bone variability and the effects of tooth loss (Kingsmill and Boyde, 1998; Schwartz-Dabney and Dechow, 2002), there are no data available so far on the macro- and micro-mechanical properties of growing mandibular bone.

The objectives of this study were to validate the usefulness of ultrasonic measurements for investigating age-related structural and mechanical changes in the growing mandible, to quantify bone maturation, and to assess the interrelationships between three defined populations of female pigs of different ages (piglets, immature sows, mature sows) with regard to speed of sound (SOS), elastic modulus, breaking strength, bone mineral density (BMD), surface hardness, and the water content.

Materials and methods

The mandibles of 30 freshly slaughtered female pigs (German Landschwein) were selected to yield three homogeneous age groups of 10 specimens each (Table 1). After dissection of the soft tissues, the specimens were frozen and stored at –18°C. They were thawed at room temperature for measurement.

Buccal cortical bone specimens were taken from the tooth-bearing part of the mandibular body at a distance of 5 mm from the mandibular inferior border in the piglets, 10 mm in the immature sows, and 15 mm in the mature sows. To ensure sample compatibility, the first primary molar or the first molar was chosen as the anterior, and the last molar as the posterior landmark (Figure 1). They were cut into rectangular blocks using a precision saw (Diadisc 5200, Mutronic, Rieden, Germany): 25 mm long in the piglets, and 35 mm in the immature and mature sows. All specimens were 10 mm in width, with a thickness ranging from 2.5 to 6 mm. Additional specimens of 10 × 10 mm were taken from the adjacent region to be used for hardness testing.

Table 1 Age and weight distribution of the animal groups.

	Age (months)	Weight (kg)
Group 1: piglets (n = 10)	2–3	30–40
Group 2: immature sows (n = 10)	4–6	≈ 100
Group 3: mature sows (n = 10)	18–24	< 200

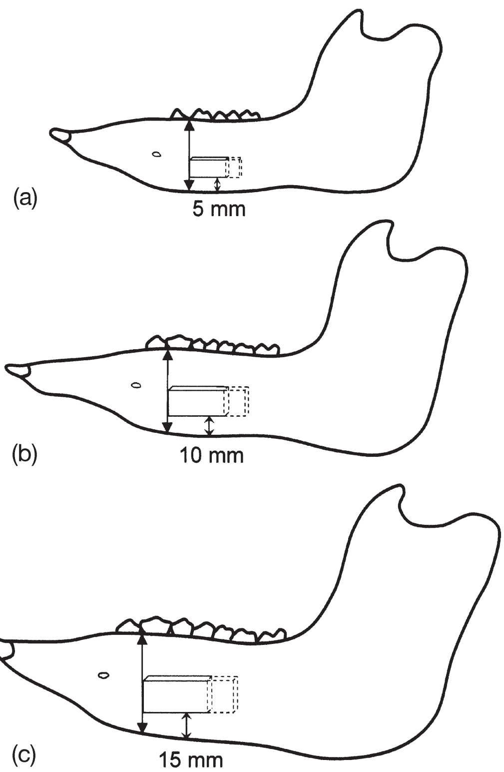

Figure 1 Schematic drawing showing the different mandibles and specimen preparations. (a) Piglet, (b) immature sow, (c) mature sow.

SOS was assessed in three orthogonal directions (antero-posterior, apico-occlusal, bucco-lingual) with a commercially available instrument (USD 10, Krautkrämer; Hürth, Germany) with pulsed ultrasound at a frequency of 2 MHz. The measurements were repeated three times with intermediate repositioning. The emitter and receiver were placed in immediate contact with the specimens. Acoustic coupling was improved by using a coupling gel (Aquasonic 100, Parker Laboratories, Orange, North Carolina, USA). Crosshead speed was calculated from the distance between the transmitter and the receiver, divided by the measured transit time.

BMD was measured by quantitative computed tomography using the Densiscan 1000 (Scanco Medical, Zurich, Switzerland). Each specimen was placed in a plexiglass container filled with Ringer's solution. The specimens were scanned in a bucco-lingual direction at four sites, at 3 mm intervals, beginning 1 mm from the specimen's anterior. Slice thickness was 1 mm, pixel size 0.088 × 0.088 mm. BMD was given in g/cm^3.

The bony tissue structure was determined by microcomputed tomography (μCT) with one specimen scanned from each group (Scanco Medical). The specimens were positioned in a plexiglass container and scanned over a length of 15 mm at a voxel size of 0.034 × 0.034 × 0.034 mm.

Breaking strength and apparent elastic modulus were assessed in a three-point bending test to failure using the Instron 4302 test rig (Instron Ltd, High Wycombe, Bucks, UK). The load of 10 kN was applied in a bucco-lingual direction using a constant crosshead speed for all samples.

The water content was determined using a standard lyophilisator (alpha 2–4 Christ Lyophilisator, Kühner, Aargau, Switzerland). The weight was determined to the nearest 0.01 g before and after lyophilization under a vacuum, at a temperature of –80°C and a pressure of 1.8 mbar.

The separate bone specimens of 10 × 10 mm were wet polished on a rotary wheel using 4000 grit paper, subsequently polished with 3 μm diamond paste, and then cleaned in an ultrasonic cleaner for 3 minutes. Micro-indentation hardness testing was determined using the Vickers' method with an optical hardness tester (Opitur, Göttfert, Buchen, Germany). The diamond's pyramid diagonal of the indentation was measured microscopically. The following formula was used to calculate bone hardness: $HV = 1854.4*L/d^2$; where L is the load in grams and d is the length of the indentation diagonal in μm. The Vickers' test was used with a load of 1961 N at three randomly selected sites on each specimen (Müller, 1970; Rahn *et al.*, 1976).

Statistical analysis

The data were statistically evaluated using the Kruskal–Wallis test and multiple regression analysis. Spearman correlation coefficients were calculated. The null hypothesis was tested at a level of significance of $P < 0.01$ and/or $P < 0.001$. Data were analysed at the Centre of Medical Biometrics and Medical Informatics at the University of Freiburg using the statistical program SAS 6.12 (Statistic Analysis System Institute, Cary, North Carolina, USA).

Results

SOS increased significantly ($P < 0.02$) with age across all groups in all three directions measured. The mean and standard deviation of the SOS measurements for each age group are presented in Table 2.

Whereas SOS, BMD ($P < 0.001$), Vickers' hardness ($P < 0.001$) and breaking strength ($P < 0.001$) increased significantly across all groups, bone water content decreased significantly ($P < 0.001$). The elastic modulus

Table 2 Comparison of the speed of sound (SOS) in m/seconds results in the different age groups for the different orientations (n = 10/age group).

	SOS antero-posterior		SOS apico-occlusal		SOS bucco-lingual	
	Mean	SD	Mean	SD	Mean	SD
Piglets	3486	210	2186	319	2729	101
Immature sows	3806	233	2841	269	2947	271
Mature sows	3951	220	3105	84	3120	479

SD, standard deviation.

increased consistently from the piglets to immature sows and mature sows, but did not reach significance (Figure 2a–h). Spearman correlation coefficients are shown in Table 3. Multiple regression analysis showed that BMD (r^2 = 69.9 per cent), water content (r^2 = 69.4 per cent) and Vickers' hardness (r^2 = 64.6 per cent) were moderate predictors for changes in SOS in the apico-occlusal direction. The increase in SOS in the antero-posterior direction was explained by BMD (r^2 = 61.9 per cent), breaking strength (r^2 = 60.4 per cent) and Vickers' hardness (r^2 = 56.1 per cent). Changes in SOS in the bucco-lingual direction could not be adequately explained by the structural and mechanical parameters of the bone investigated. The μCT images reflected changes in architectural organization during maturation of the mandibular cortical bone (Figure 3a–c). The cortical bone of the piglets was more porous and less compact than that of the older sows. In the immature sow, the bone seemed more compact and uniform, whereas in the mature sow a more porous cortex was found, which may reflect latent osteoporosis.

Discussion

In orthodontics, the extent of tooth movement depends on the ability of cells to respond to mechanical forces. Continuous micro- and macro-architectural changes occur simultaneously during bone maturation during growth and ageing. There are no valid clinical diagnostic techniques for following bone maturation in children for orthodontic purposes without risking unjustifiable side-effects.

SOS might be one clinical method of investigating bone quality and bone maturation in adolescents because it reflects age-related changes in growing bone (Nemet *et al.*, 2001; Barkmann *et al.*, 2002). In the present study, parameters that change significantly during growth and show a significant correlation with SOS were investigated. SOS measurements at the calcaneus in children show age dependency and a correlation with increasing bone density (Jaworski *et al.*, 1995). However, SOS measurements at locations of mainly cortical bone produce more consistent results, even detecting sex differences (Harten *et al.*, 1997). Changes in SOS in the thumb and patella increase with age after puberty and peak at 20–25 years of age (Schönau *et al.*, 1994).

Correlations between ultrasound velocity and the age-dependent structural and mechanical properties of bone have been found, particularly in research on osteoporosis. A linear correlation between SOS and BMD in calcaneum was shown by Tavakoli and Evans (1991) and Han *et al.* (1996). Bouxsein and Radloff (1997) reported a moderate to strong relationship between SOS and BMD in intact feet. Greenfield *et al.* (1975, 1981) showed that this relationship can also be applied to cortical bone. BMD and the elastic modulus of healthy patients' bone can be calculated from SOS and bone mineral content derived from measurements at the proximal radius. Accordingly, SOS may give an indication of BMD, and thus could be used *in vitro* and *in vivo* as an additional method to estimate BMD.

In the present study, the cortical bone of three different age groups of female pigs was investigated. The pig race used starts menstruating at 4–6 months of age, at an average weight of 100–120 kg. Regarding sexual maturation, it can be assumed that the piglets represent pre-puberty, the immature sow group puberty and the mature sow group post-puberty.

Table 3 Spearman correlation coefficient (n = 30).

	SOS ap	SOS ao	SOS bl	BMD	EM	HV	WC	BS
SOS ap	1.00	0.670	0.549	0.762	0.608	0.726	–0.745	0.722
SOS ao		1.00	0.685	0.851	0.373	0.797	–0.877	0.742
SOS bl			1.00	0.610	0.486	0.471	–0.582	0.539
BMD				1.00	0.520	0.884	–0.952	0.863
EM					1.00	0.422	–0.474	0.733
HV						1.00	–0.826	0.684
WC							1.00	–0.888
BS								1.00

SOS, speed of sound; ap, antero-posterior; ao, apico-occlusal; bl, bucco-lingual; BMD, bone mineral density; EM, elastic modulus; HV, Vickers' hardness; WC, water content; BS, bending strength.

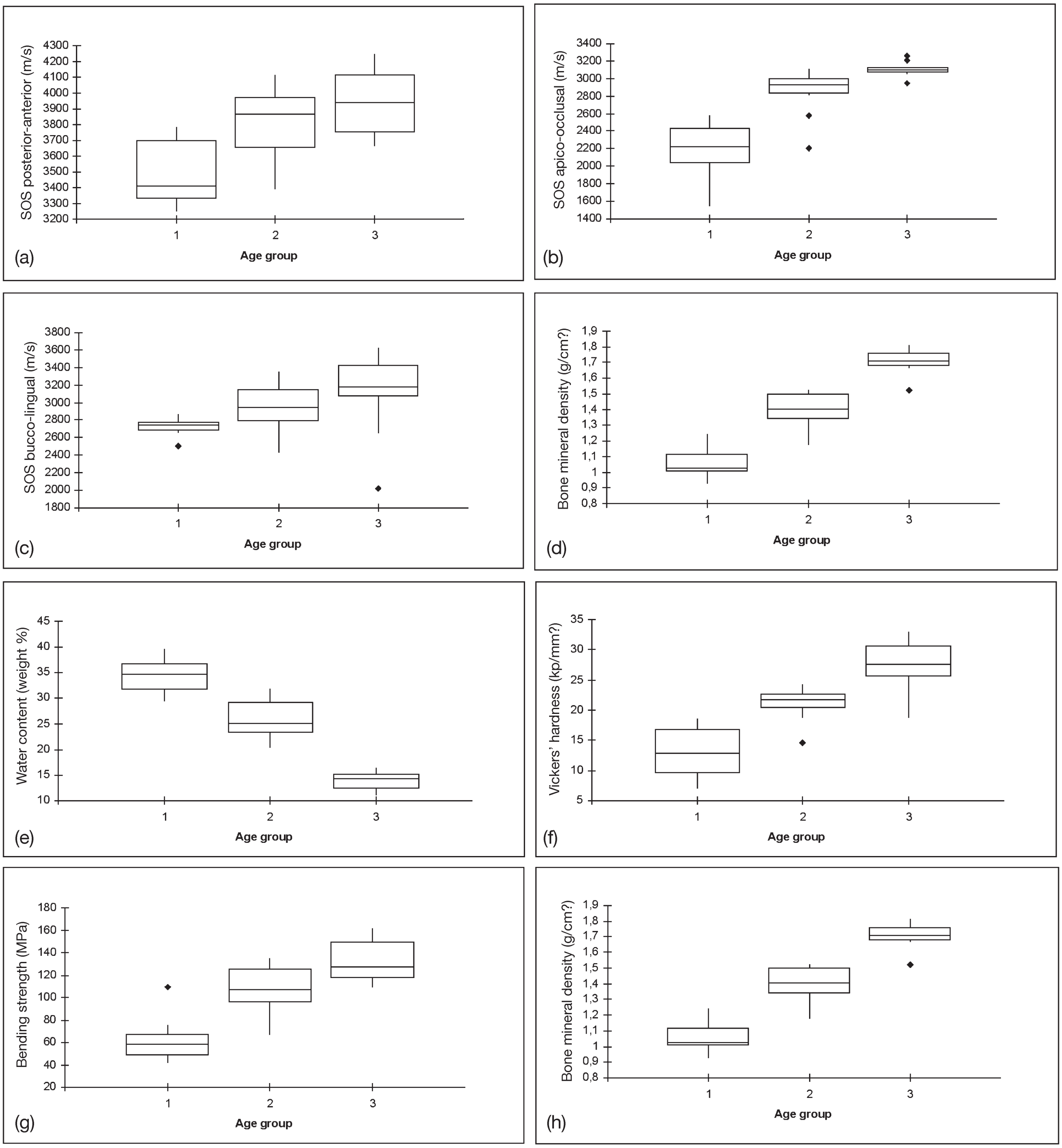

Figure 2 Dependence of the parameters investigated on the age of the animals. (a) Speed of sound (SOS) antero-posterior (m/second), (b) SOS apico-occlusal (m/second), (c) SOS bucco-lingual (m/second), (d) bone mineral density (g/cm3), (e) water content (weight proportion %), (f) Vickers' hardness (kp/mm2), (g) bending strength (MPa), (h) elastic modulus (GPa). Age group 1 = piglets, 2 = immature sows, 3 = mature sows. The diamond indicates outliers.

There is a considerable discrepancy between the elastic modulus data derived from the present study and other investigators' results, which were generally higher. A possible reason for this may be the scattering of the values in the three age groups. In addition to these differences noted in the bone density of piglets, the bone density of immature and mature sows had to be considered. Such differences could result from the fact that the animals had been fed differently, according to their purpose in life, e.g. breeding. Furthermore, the geometry of the specimens used in the mechanical testing must be considered. Because of the anatomical site chosen, some specimens showed irregularities, such as in cancellous parts or nerve channels. No further preparation was carried out so as to keep the bone as close to the original anatomical conditions. A decrease in bone water content during growth was also described by Jonsson *et al.* (1985). There was a simultaneous

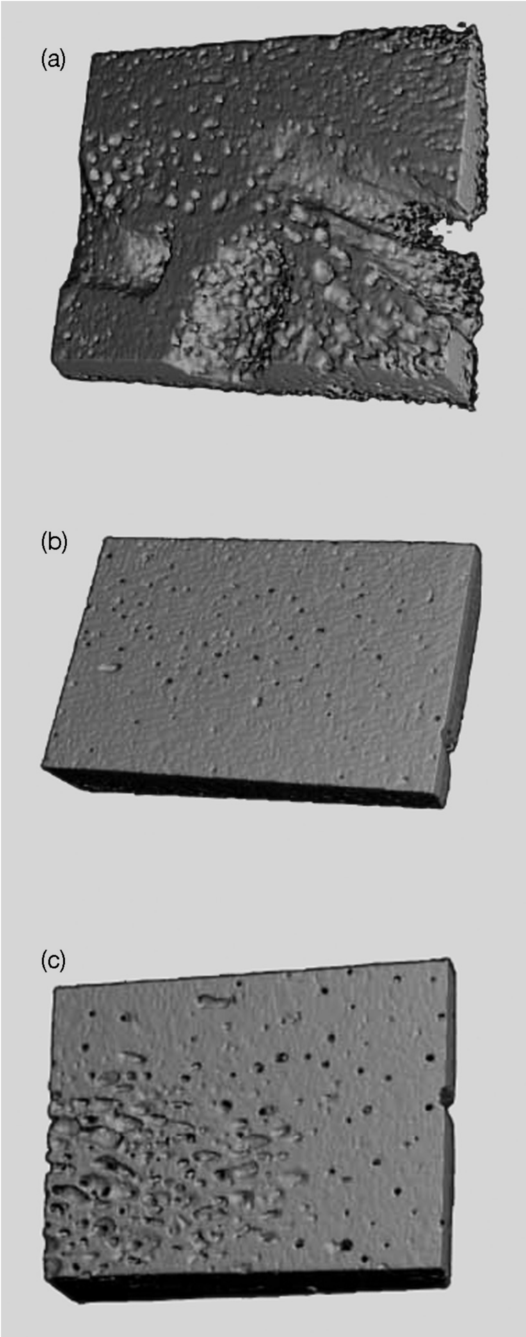

Figure 3 Microcomputed tomographic images of cortical bone specimens of the mandible of (a) a piglet, (b) an immature sow, (c) a mature sow. Bucco-lingual views, magnification ×5.5.

increase in bone tissue followed by changes in the bone's physical properties, for example, in bending strength and elastic modulus. In addition to a significant decrease in bone water content and the related changes in mechanical parameters, there was a corresponding increase in SOS as ageing progressed.

Surface hardness of cortical bone was thoroughly investigated by Weaver (1966), who found that Vickers' hardness correlated with the state of mineralization in his specimens. He also found an increase in surface hardness corresponding to the advance in mineralization during skeletal maturation. This confirms the results of the present study concerning the age-dependent changes in Vickers' hardness throughout growth.

Maturation of the mandible implies changes in its architectural organization, in bone tissue composition, and in the mechanical properties of cortical bone. Age-related changes in bone tissue during maturation can be determined by mechanical testing and measurement of water content, both procedures that cannot be performed in a non-destructive manner. Radiological procedures, which depict these changes in a similar manner, should be kept to a minimum in individuals. Thus, the non-destructive method of using ultrasound appears attractive. SOS in cortical bone is age-dependent with statistical significance, and correlates well with various structural and mechanical parameters. However, its changes cannot be fully explained by the variables investigated, thus precluding the prediction of one parameter on the basis of another in the same individual. This may account for the disparity between the various published classifications. A future study design should focus on more clinical parameters to validate the methods for clinical use.

Conclusion

In vitro SOS measurements reflect various overall, age-dependent changes in cortical bone, and could eventually be used to estimate bone maturity. Further clinical investigations are needed to determine the clinical significance of these results, especially the problems arising from more complex geometrical properties of test sites, e.g. influences of bicortical and cancellous bone, tooth roots and tooth germs, and overlying soft tissue.

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